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ASIC-like, proton-activated currents in rat hippocampal neurons

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The expression of mRNA for acid sensing ion channels (ASIC) subunits ASIC1a, ASIC2a and ASIC2b has been reported in hippocampal neurons, but the presence of functional hippocampal ASIC channels was never assessed. We report here the first characterization of ASIC-like currents in rat hippocampal neurons in primary culture. An extracellular pH drop induces a transient Na^+ current followed by a sustained non-selective cation current. This current is highly sensitive to pH with an activation threshold around pH 6.9 and a $pH_{0.5}$ of 6.2. About half of the total peak current is inhibited by the spider toxin PcTX1, which is specific for homomeric ASIC1a channels. The remaining PcTX1-resistant ASIC-like current is increased by 300 μM Zn^{2+} and, whereas not fully activated at pH 5, it shows a $pH_{0.5}$ of 6.0 between pH 7.4 and 5. We have previously shown that Zn^{2+} is a co-activator of ASIC2a-containing channels. Thus, the hippocampal transient ASIC-like current appears to be generated by a mixture of homomeric ASIC1a channels and ASIC2a-containing channels, probably heteromeric ASIC1a+2a channels. The sustained non-selective current suggests the involvement of ASIC2b-containing heteromeric channels. Activation of the hippocampal ASIC-like current by a pH drop to 6.9 or 6.6 induces a transient depolarization which itself triggers an initial action potential (AP) followed by a sustained depolarization and trains of APs. Zn^{2+} increases the acid sensitivity of ASIC channels, and consequently neuronal excitability. It is probably an important co-activator of ASIC channels in the central nervous system.

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H^+-gated cation channels are present in sensory neurons and in neurons of the central nervous system (CNS). Several H^+-gated cation channel subunits (ASIC, acid sensing ionic channels) have been cloned and functionally expressed so far: ASIC1a (Waldmann *et al.* 1997*b*), ASIC1b (Chen *et al.* 1998), ASIC2a (Price *et al.* 1996; Waldmann *et al.* 1996), ASIC2b (Lingueglia *et al.* 1997), ASIC3 (Waldmann *et al.* 1997*a*; de Weille *et al.* 1998; Babinski *et al.* 1999). Very recently, another putative ASIC subunit (ASIC4) was identified which seems not to be activated by acidic pH (Akopian *et al.* 2000; Grunder *et al.* 2000).

Functional ASIC channels are thought to be tetrameric assemblies of ASIC subunits (Coscoy *et al.* 1998; Waldmann *et al.* 1999). Both homomeric and heteromeric ASIC channels can be formed (Bassilana *et al.* 1997; Waldmann & Lazdunski, 1998; Waldmann *et al.* 1999; Babinski *et al.* 2000), thus contributing to the functional diversity of neuronal ASIC-like channels (Grantyn & Lux, 1988; Ueno *et al.* 1992; Varming, 1999; Escoubas *et al.* 2000; Sutherland *et al.* 2000*a*).

Some ASIC subunits are specifically expressed in sensory neurons, like ASIC1b (Chen *et al.* 1998) and ASIC3 (Waldmann *et al.* 1997*a*; Voilley *et al.* 2001), whereas ASIC1a (Bassilana *et al.* 1997; Olson *et al.* 1998; Voilley *et al.* 2001), ASIC2a (Price *et al.* 1996, 2000; Bassilana *et al.* 1997; Lingueglia *et al.* 1997; Garcia-Anoveros *et al.* 2001), ASIC2b (Lingueglia *et al.* 1997) and ASIC4 (Akopian *et al.* 2000; Grunder *et al.* 2000) are also found in central neurons.

In sensory neurons, ASIC currents are thought to play an important role in nociception during a tissue acidosis and inflammation (Olson *et al.* 1998; Waldmann & Lazdunski, 1998; Benson *et al.* 1999; Kress & Zeilhofer, 1999; Waldmann *et al.* 1999; Sutherland *et al.* 2000*a*,*b*; Voilley *et al.* 2001), and ASIC2a has been proposed to participate in touch sensation (Price *et al.* 2000; Garcia-Anoveros *et al.* 2001).

The role of ASIC1a, ASIC2a, ASIC2b and ASIC4 in central neurons (Price *et al.* 1996; Waldmann *et al.* 1996; Bassilana *et al.* 1997; Garcia-Anoveros *et al.* 1997) remains to be established. An acidosis accompanies brain ischaemia or epilepsy, and ASIC currents might contribute to the associated neuronal death. However, pH fluctuations also occur in normal brain function. Several studies with brain slices indicate that neuronal activity gives rise to significant and rapid changes in extracellular pH (Krishtal *et al.* 1987; Chesler & Kaila, 1992). Because of the limited spatial and temporal resolution of pH microelectrodes used in those

studies, the global pH variations that have been reported may underestimate the actual pH changes occurring within or in the vicinity of the synaptic cleft (Chesler & Kaila, 1992). The content of synaptic vesicles is acidic (Ozkan & Ueda, 1998) and synaptic release during neuronal activity is expected to create an extracellular acidification in the vicinity of the synaptic cleft. For hippocampal neurons, an intravesicular pH of 5.7 has been measured with a pH-sensitive fluorescent probe (Miesenbock *et al.* 1998). The same study showed a transient decrease in the extracellular pH to about 6.4 after secretion of synaptic vesicles.

Synaptic vesicles of hippocampal glutamatergic neurons also contain a high amount of Zn^{2+}, particularly in the dentate granule cells and their projections, the mossy fibres (Frederickson, 1989). Vesicular Zn^{2+} is co-released with the neurotransmitter (i.e. glutamate), resulting in a transient increase of the local synaptic Zn^{2+} concentration up to 100–300 μM from resting levels below 500 nM (Assaf & Chung, 1984; Howell *et al.* 1984; Smart *et al.* 1994; Budde *et al.* 1997; Weiss *et al.* 2000). Zn^{2+} is known to exert a variety of effects on ion channels, among which are AMPA and NMDA receptors, and was thus proposed to be a neuromodulator of the excitatory glutamatergic synapse (Harrison & Gibbons, 1994; Smart *et al.* 1994; Henze *et al.* 2000; Vogt *et al.* 2000).

In order to understand the function of the brain ASIC subunits, particularly in hippocampus which is involved in memory processes and in the physiopathology of ischaemia (Schmidt-Kastner & Freund, 1991; Henze *et al.* 2000), we recorded and characterized the molecular constitution of the ASIC-like current in hippocampal neurons. We previously showed that Zn^{2+} potentiates the activation of ASIC2a-containing ASIC channels (Baron *et al.* 2001). We demonstrate here that the hippocampal ASIC-like current is coactivated by Zn^{2+} resulting in an increase in neuronal excitability.

METHODS

Primary cultured hippocampal neurons

Primary cell cultures derived from rat embryonic hippocampi, containing mainly neurons over astrocytes, were established as described previously (Goslin & Banker, 1991; Lauritzen *et al.* 1997). In brief, 18- to 19-day-pregnant Wistar rats were stunned and killed by decapitation, according to national and institutional guidelines. Embryos were removed and hippocampi were dissected, incubated in 0.125% trypsin for 35 min at 37°C, and dissociated mechanically. Cells were plated in modified Eagle's medium (MEM) containing 10 % dialysed inactivated horse serum (Sigma), 6 g l^{-1} glucose, 50 U ml^{-1} penicillin, and 50 μg ml^{-1} streptomycin. Cells were plated (day 0) at a density of ~750 000 cells per 35-mm poly-L-lysine-coated tissue culture plates (Falcon). After 48 h, the culture medium was replaced with serum-free MEM with 2 % B27 supplement (Gibco) and 6 g l^{-1} glucose, and kept in 95 % air–5 % CO_2 at 37 °C. Cells were used for electrophysiological recordings 7–20 days after plating. Neurons with triangular-shaped cell bodies, a typical feature of pyramidal neurons, were selected for recording.

Patch-clamp recordings of hippocampal ASIC-like currents

Ion currents were recorded using the whole-cell patch-clamp technique (Hamill *et al.* 1981). Data were sampled at 500 Hz and low-pass filtered at 3 kHz using pCLAMP 8 software (Axon Instruments, USA). The statistical significance of differences between sets of data was estimated by the single-sided Student test. The pipette solution contained (mM): KCl 140, NaCl 5, $MgCl_2$ 2, EGTA 5, K_2ATP 2, Hepes 10 (pH 7.35), and the bath solution contained (mM): NaCl 150, KCl 5, $MgCl_2$ 2, $CaCl_2$ 2, glucose 10 mM, Hepes 10 (pH 7.45). CNQX 20 μM, kynurenic acid 10 μM, $MgCl_2$ 7 mM and bicuculline 10 μM were added in order to inhibit glutamate- and GABA-induced currents. MES or acetate were used instead of Hepes to buffer bath solution pH ranging from 6 to 5, and from 4.5 to 3, respectively. $ZnCl_2$ was added to the bath solution at 300 μM (Baron *et al.* 2001). Changes in extracellular pH were induced by shifting one out of six outlets of a microperfusion system in front of the cell. Experiments were carried out at room temperature (20–24 °C). Bovine serum albumin (0.1 %) was added in extracellular solutions containing the spider toxin PcTX1 (Escoubas *et al.* 2000) to prevent its adsorption to tubing and containers. Amiloride, CNQX, bicuculline, capsaicin, capsazepine and kynurenic acid were all from Sigma.

RESULTS

ASIC-like current of primary cultured hippocampal neurons

The expression of ASIC1a and ASIC2a was previously studied in hippocampal neurons by *in situ* hybridization. A high level of overlapping expression of the two subunits was particularly found in the pyramidal neurons of CA1 and CA3 subfields of the hippocampus (Bassilana *et al.* 1997; Waldmann *et al.* 1997*a*,*b*). However, the presence of functional hippocampal ASIC channels was never assessed. When the neurons were held at −50 mV, acidification of the extracellular medium triggered a transient inward current that was frequently followed by a plateau phase (Fig. 1*A* and *C*). At −50 mV, a drop to pH 5 induced a mean peak current of 17.15 ± 1.86 pA pF^{-1} and a mean plateau current of 1.41 ± 0.33 pA pF^{-1} (cell capacitance: 39.8 ± 2.7 pF, n = 52). The *I–V* curve shows that the peak transient current (Fig. 1*A* and *B*, ○) is highly selective for Na^+ (E_{rev} (reversal potential) = +80 mV) whereas the plateau current was non-selective (E_{rev} = +15 mV; Fig. 1*A* and *B*, ●).

The hippocampal ASIC-like peak current showed a high sensitivity to pH, with an activation threshold around pH 6.9 (Fig. 1*C* and *D*). Between pH 7.4 and 5, the activation of the peak current could be fitted by a sigmoidal curve, with a half-maximal activation at pH 6.2 and a Hill slope factor (n_H) of 1.48 (Fig. 1*D*). These activation properties are intermediate between those of heterologously expressed homomeric ASIC1a and heteromeric ASIC1a+2a currents ($pH_{0.5}$ = 6.4 and n_H = 1.65 for ASIC1a, $pH_{0.5}$ = 5.5 and n_H = 1.10 for ASIC1a+2a (Baron *et al.* 2001; Fig. 1*D*). This suggests that the hippocampal ASIC-like current flows through a mixture of different channels. This view is

supported by the fact that the current is not fully activated at pH 5 and can be further increased at pH 4.

Pharmacological properties of hippocampal ASIC-like current

The peak current was fully inhibited by 500 μM amiloride, a known blocker of ASIC channels (Linguegliaet *et al.* 1993; Waldmann *et al.* 1997b, 1999), whereas the plateau current was not fully suppressed (Fig. 2*A*). The hippocampal ASIC-like current was not inhibited by 10 μM capsazepine, and 100 μM capsaicin evoked only a small inward current in hippocampal neurons (0.7 ± 0.3 pA pF^{-1}, $n = 5$, not shown), showing that a contamination of the ASIC-like current by the pH-activated VR1 current (Tominaga *et al.* 1998; Szallasi & Di Marzo, 2000) can be ruled out. In the majority (80 %) of the recorded neurons, the hippocampal ASIC-like peak current induced at pH 6 was inhibited by 46 ± 8 % ($n = 12$) by the tarantula toxin PcTX1, a specific blocker of homomeric ASIC1a channels (Escoubas *et al.* 2000) (Fig. 2*B*), whereas the inhibition was complete in the remaining 20 % of cells. This result shows that the hippocampal ASIC-like current is due to a mixture of homomeric ASIC1a channels and at least one other PcTx1-resistant ASIC channel.

Effect of Zn^{2+} on hippocampal ASIC-like current

Previous work with recombinant channels has shown that Zn^{2+} co-applied with acidic pH increases the amplitude of ASIC2a-containing currents (Baron *et al.* 2001). We tested the effect of Zn^{2+} on the hippocampal ASIC-like current with two purposes in mind. The first was to use Zn^{2+} as a pharmacological tool to reveal the involvement of ASIC2a subunits in hippocampal ASIC assemblies. The second was to establish a possible physiological role for Zn^{2+} in the activation of these channels. In 80 % of the recorded cells, 300 μM Zn^{2+} increased the peak hippocampal ASIC-like current, with no effect on the sustained current (Fig. 3*A*). The PcTX1-resistant peak current was more sensitive to Zn^{2+} than the whole ASIC-like current (Fig. 3*B*), consistent with the fact that the homomeric ASIC1a current is insensitive to Zn^{2+} (Baron *et al.* 2001). In the presence of PcTX1, 300 μM Zn^{2+} increased the amplitude of the peak ASIC-like current by a factor of 4.74 ± 0.73 ($n = 4$) at pH 6.9, by a factor of 1.86 ± 0.19 ($n = 6$) at pH 6, and by a

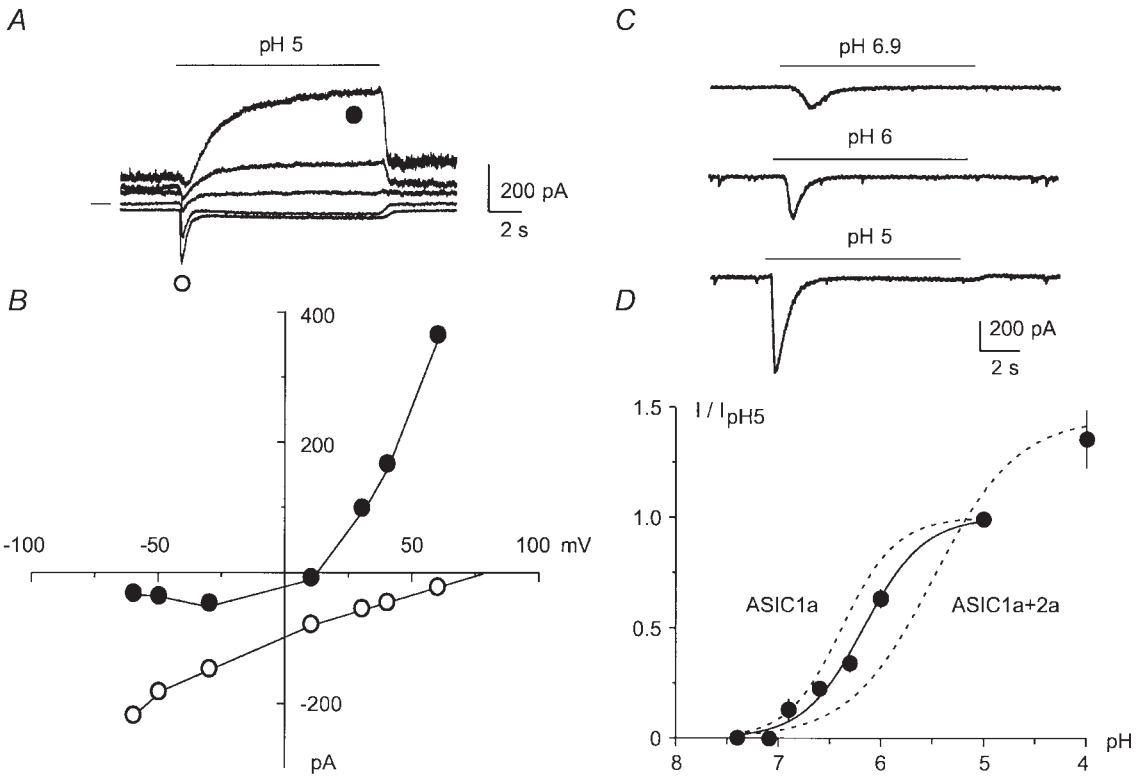

Figure 1. ASIC-like current in hippocampal neurons

A, hippocampal ASIC-like currents activated at pH 5 and recorded at −60, −30, +10, +30 and +60 mV. The 0 pA current level is indicated on the left. *B*, current–potential relationship of hippocampal ASIC-like current obtained from traces shown in *A*. ○, peak current; ●, plateau current. *C*, hippocampal ASIC-like currents induced by pH 6.9, 6 and 5. Currents were recorded at −50 mV. *D*, pH-dependent activation of the hippocampal ASIC-like current. Current amplitude was expressed as a fraction of the current induced by pH 5 ($I/I_{pH\,5}$), and plotted as mean ± S.E.M., n ranging from 8 to 18. Between pH 7.4 and 5, data could be fitted by a sigmoidal curve, showing a $pH_{0.5}$ of 6.2 and a Hill coefficient of 1.48. The dashed curves represent the pH-dependent activation of the homomeric ASIC1a and of the heteromeric ASIC1a+2a (1:1) currents expressed in *Xenopus* oocytes (Baron *et al.* 2001).

factor of 1.33 ± 0.11 ($n = 4$) at pH 5. These values are similar to those obtained for the ASIC1a+2a current expressed in *Xenopus* oocytes or in COS cells (Baron *et al.* 2001). The few Zn^{2+}-insensitive currents were highly (90–100 %) inhibited by PcTX1, and would thus correspond to homomeric ASIC1a currents.

To investigate the molecular association involved in hippocampal Zn^{2+}-sensitive ASIC-like current, we measured the pH sensitivity of the PcTX1-resistant Zn^{2+}-sensitive ASIC-like current (Fig. 3*C*, ○) and compared it with the pH sensitivity of the PcTx1-sensitive Zn^{2+}-insensitive ASIC-like current (Fig. 3*C*, ●). The PcTx1-sensitive Zn^{2+}-insensitive current showed a $pH_{0.5}$ of 6.3 with a maximal activation at pH 5, which would be expected from homomeric ASIC1a current. In contrast, the PcTX1-resistant Zn^{2+}-sensitive current was not maximal at pH 5 and greatly increased at pH 4. Between pH 7.4 and 5, a $pH_{0.5}$ of 6.0 was obtained, a value significatively lower than that for both the PcTX1-sensitive Zn^{2+}-insensitive current (ASIC1a-like) or the whole ASIC-like current. These results support the suggestion that ASIC2a-containing heteromers are involved between pH 7.4 and 5, whereas the further increase in current at pH 4 could be mainly due to homomeric ASIC2a channels.

The Zn^{2+} sensitivity of the peak hippocampal ASIC-like current thus appears to be conferred by a PcTX1-resistant current, probably flowing through ASIC1a+2a channels.

Effect of Zn^{2+} and ASIC-like current on the membrane potential of hippocampal neurons

Membrane potential variations have been recorded in the current-clamp mode. Hippocampal neurons had a resting potential of −49.8 ± 2.0 mV ($n = 29$) which usually prevented the triggering of spontaneous action potentials (APs) (Fig. 4*A* and *D*). Acidification of the extracellular medium induced a biphasic depolarization with a transient phase and a plateau (Fig. 4*Aa–c*), compatible with kinetics of the hippocampal ASIC-like current (Fig. 4*Ad*). The transient depolarizations induced by pH shifts from 7.4 to 6.6 or 5 triggered an initial AP (Fig. 4*Ab* and *c*, enlargements shown), whereas the threshold of the AP was not reached by the transient depolarization induced by a pH drop to 6.9 (Fig. 4*Aa*). However, spontaneous AP trains could often be recorded during the plateau of the depolarization induced by pH drops to 6.9 or to 6.6 (Fig. 4*Aa* and *b*), whereas lower pH values (pH 5 in Fig. 4*Ac*) induced a sustained depolarization to around −20 mV but did not produce any AP. This can be easily explained by an inactivation of the voltage-sensitive Na^+ channels by the sustained depolarization, and the prevention of any AP triggering.

The transient peak of the acid-induced depolarization at pH 5 (46.4 ± 2.3 mV; $n = 12$) corresponds to a membrane potential close to 0 mV, whereas the maximal depolarization induced by a pH drop from 7.4 to 6.9 (10.6 ± 4.0 mV; $n = 9$) would bring the membrane potential to around −40 mV (Fig. 4*B*). Figure 4*C*, showing the relationship between the ASIC current density and the membrane depolarization induced by the same pH drop on the same neuron, illustrates the fact that 25 % of the maximal ASIC current could induce 50 % of the maximal depolarization. Thus, a slight drop of the extracellular pH and a submaximal activation of ASIC channels may cause important changes in the excitability of hippocampal neurons.

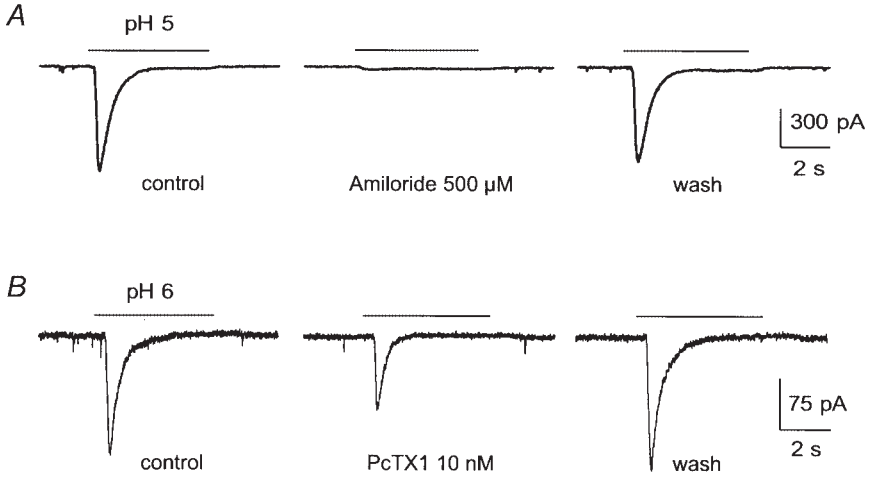

Figure 2. Pharmacological properties of the hippocampal ASIC-like current

A, reversible inhibition of hippocampal ASIC-like current by 500 μM amiloride. *B*, reversible inhibition of hippocampal ASIC-like current by 10 nM of the toxin PcTX1. Currents were recorded at −50 mV. Amiloride and PcTX1 were given before and during the pH drop.

Zn^{2+} potentiates the hippocampal ASIC-like current. It also increases the submaximal acid-induced transient depolarizations. Figure 4*D* shows the effect of 300 μM Zn^{2+} co-applied with pH 6.9. In the absence of Zn^{2+}, APs were only recorded during the plateau phase of the depolarization induced by pH 6.9 (Fig. 4*Da*), whereas, in the presence of 300 μM Zn^{2+}, APs were also triggered during the increased transient depolarization (Fig. 4*Db*). The depolarization induced by a pH drop to 5 was not significantly modified by Zn^{2+} (not shown). This was expected considering that a

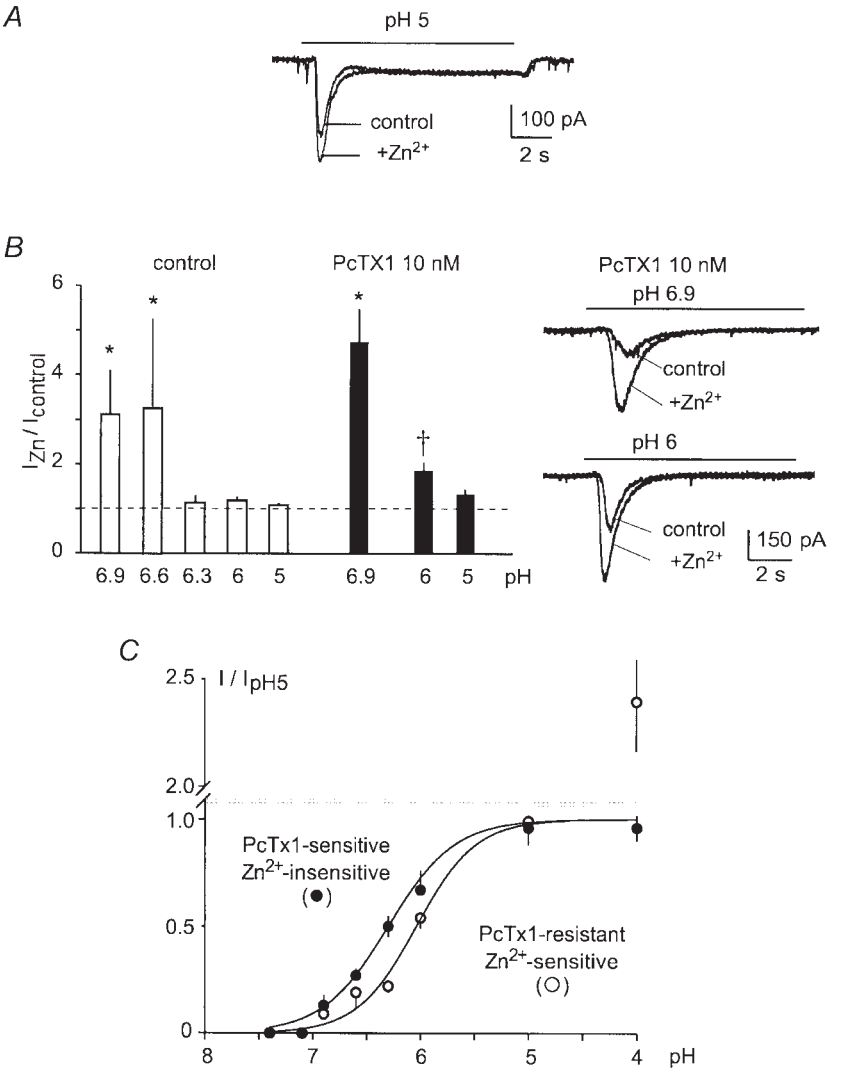

Figure 3. Effect of Zn^{2+} on the hippocampal ASIC-like current

A, 300 μM Zn^{2+} co-applied with acidic pH increases the amplitude of the peak hippocampal ASIC-like current without affecting the plateau phase. Currents were recorded at −50 mV. *B*, effect of 300 μM Zn^{2+} on hippocampal ASIC-like currents induced by pH ranging from 6.9 to 5. Current amplitude ratio ($I_{Zn}/I_{control}$) was measured for each pH value, in the absence (□) and in the presence of 10 nM PcTX1 (■), and plotted as mean ± S.E.M., *n* ranging from 4 to 22; * significantly different from corresponding pH 5 ratio ($P < 0.05$); † Significantly different from corresponding control (no PcTX1) pH ratio ($P < 0.005$). Currents were recorded at −50 mV. On the right side on the histogram, original current traces are shown to illustrate the effect of Zn^{2+} on hippocampal ASIC-like current induced by pH 6.9 (top) and pH 6 (bottom) in the presence of PcTX1 (holding potential: −50 mV). *C*, pH-dependent activation of the PcTX1-sensitive Zn^{2+}-insensitive ASIC-like current (●) and of the PcTX1-resistant Zn^{2+}-sensitive ASIC-like current (○). Data for PcTX1-resistant ASIC-like current were obtained from the same experiments as in *B*. Data for PcTX1-sensitive Zn^{2+}-insensitive ASIC-like current were obtained from neurons (20 % of total recorded neurons) showing an ASIC-like current highly sensitive to PcTX1 (90–100 % inhibited) and not potentiated by Zn^{2+}. Current amplitude was expressed as a fraction of the current induced by pH 5 (I/I_{pH5}), and plotted as mean ± S.E.M., *n* ranging from 4 to 18. Between pH 7.4 and 5, data could be fitted by sigmoidal curves, showing a $pH_{0.5}$ of 6.3 for the PcTX1-sensitive Zn^{2+}-insensitive ASIC-like current and a $pH_{0.5}$ of 6.0 for the PcTX1-resistant Zn^{2+}-sensitive ASIC-like current.

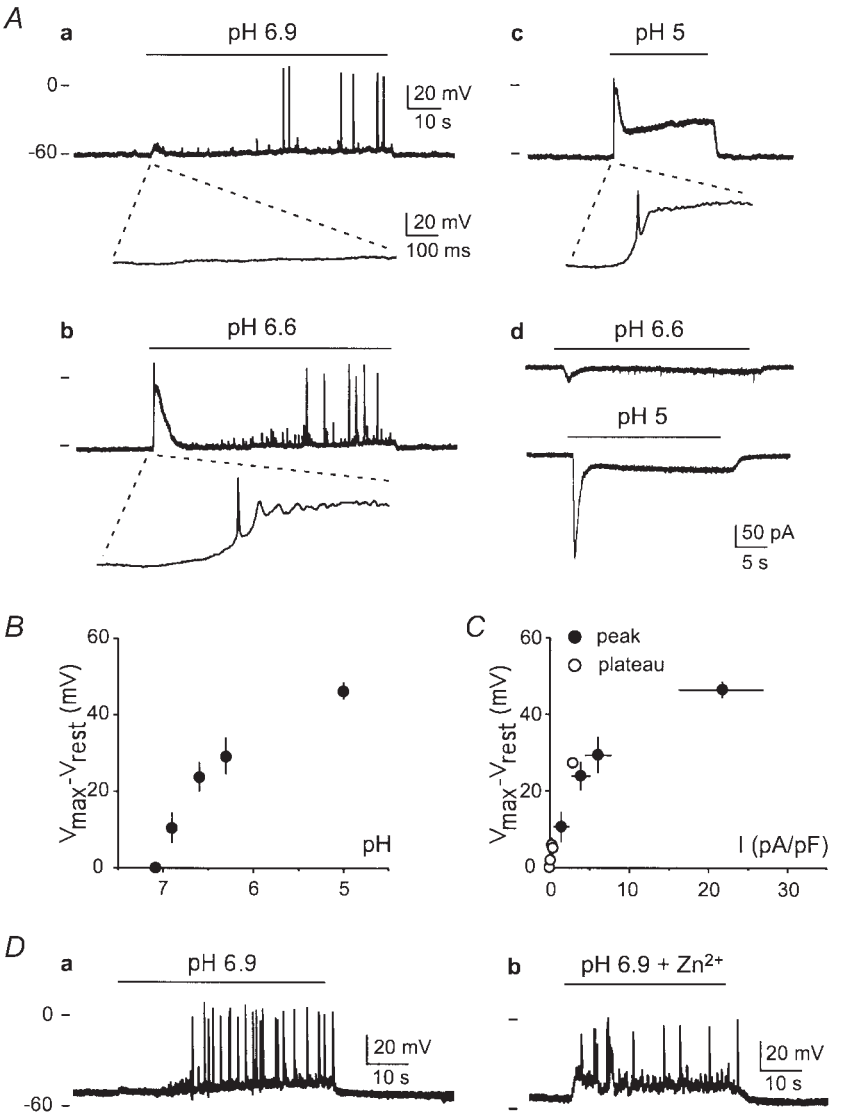

Figure 4. Effect of ASIC current activation on membrane potential of hippocampal neurons

A, membrane depolarization induced by pH 6.9 (*a*), pH 6.6 (*b*) and pH 5 (*c*) in a single neuron. The initial acid-induced depolarization and AP is shown on a higher scale (100-fold) under each recording. Membrane potential was recorded in current-clamp mode with 0 pA current. Resting potential was −63 mV. Ticks on the left side of recordings represent the 0 mV level (upper tick) and the −60 mV level (lower tick). On the same neuron, ASIC-like currents activated by pH 6.6 and 5 were subsequently recorded in voltage-clamp mode at −60 mV (*d*). *B,* mean maximal membrane depolarization induced by ASIC-like current activation ($V_{max} - V_{rest}$) as a function of extracellular pH. Recordings with a resting membrane potential (V_{rest}) between −45 and −55 mV were selected, and action potentials were excluded of measurements. Mean ± S.E.M. values are shown, *n* ranging from 3 to 12. *C,* mean maximal membrane depolarization induced by ASIC-like current activation ($V_{max} - V_{rest}$) as a function of current density. For membrane potential measurements, recordings with a resting membrane potential between −45 and −55 mV (V_{rest}) were selected, and action potentials were excluded of measurements. ASIC-like current density (pA pF^{-1}) was subsequently measured on the same neurons, at holding potential between −45 and −55 mV. Membrane potential and ASIC-like current amplitude were measured during the transient phase (●) and during the sustained plateau phase (○) for the same pH value. Mean ± S.E.M. values are shown, *n* ranging from 3 to 12. *D,* effect of 300 μM Zn^{2+} on membrane depolarization induced by ASIC-like current activation. Membrane depolarization induced by pH 6.9 (*a*) and pH 6.9 + 300 μM Zn^{2+} (*b*) on a single neuron. Membrane potential was recorded in current-clamp mode with 0 pA current. Resting membrane potential was −53 mV. Ticks on the left side of recordings represent the 0 mV level (upper tick) and the −60 mV level (lower tick).

pH change of this magnitude already induced a quasi-maximal depolarization (Fig. 4*C*).

DISCUSSION

ASIC-like currents in CNS neurons

Several ASIC-like currents have been recorded in different types of central neurons. However, the available data show an important diversity of ASIC-like currents depending on the neuronal type. In mouse cerebellar granule cells, the half-maximal activation of ASIC-like current was obtained at $pH_{0.5} = 6.4$, and the current was nearly completely inhibited by PcTX1 ($IC_{50} = 0.7$ nM). These two properties suggested that the ASIC-like current mainly flows through homomeric ASIC1a channels in cerebellar granule cells (Escoubas *et al.* 2000). With a half-maximal activation at pH 6.8 (Grantyn & Lux, 1988), ASIC-like currents in rat tectal neurons also seem to flow through homomeric ASIC1a channels, although a pharmacological analysis using PcTX1 would be needed to confirm this. In rat ventromedial hypothalamic neurons (Ueno *et al.* 1992), the properties of ASIC-like currents are quite different with a threshold at pH 6.5, a maximal activation at pH 4 and $pH_{0.5} = 4.9$. These properties seem closer to those of the heteromeric ASIC1a+2a current (Baron *et al.* 2001). In mouse cortical neurons, low $pH_{0.5}$ values also suggest the involvement of heteromeric ASIC1a+2a channels (Varming, 1999). However, here again, because no pharmacological tools or antibodies were available, it was impossible to elucidate the exact molecular nature of the ASIC channels.

We report here the first characterization of ASIC-like current in hippocampal neurons. Extracellular acidification induces a biphasic current, i.e. a transient Na^+ current followed by a sustained non-selective cation current. The partial block by the ASIC1a-specific toxin PcTX1 (Escoubas *et al.* 2000), the pH dependence and the Zn^{2+} co-activation of the hippocampal current suggest that the transient current flows through a mixture of PcTX1-sensitive Zn^{2+}-insensitive homomeric ASIC1a channels and of PcTX1-resistant Zn^{2+}-sensitive ASIC2a-containing channels. The biphasic pattern of the PcTX1-resistant current activation curve (Fig. 3*C*, ○) suggests that the PcTX1-resistant current involves as least two different channel types: putative heteromeric ASIC1a+2a with a $pH_{0.5}$ of 6.0, and putative homomeric ASIC2a channels mainly responsable for the increase in current amplitude at pH 4. The pH sensitivity of heteromeric ASIC1a+2a channels is highly dependent on the stoichiometry of the two subunits. Whereas homomeric ASIC1a current shows a $pH_{0.5}$ of 6.4 and the homomeric ASIC2a shows a $pH_{0.5}$ of 4.4, the heteromeric ASIC1a+2a (1:1) current shows a $pH_{0.5}$ of 5.5 and the heteromeric ASIC1a+2a (1:2) current shows a $pH_{0.5}$ of 5.1 (Baron *et al.* 2001; A. Baron, unpublished data). Thus, the $pH_{0.5}$ of 6.0 obtained for the PcTX1-resistant Zn^{2+}-sensitive hippocampal current activated between pH 7.4 and 5 suggests that the heteromeric ASIC1a+2a channels would contain a higher proportion of ASIC1a subunits than of ASIC2a subunits. This hypothesis is supported by semi-quantitative RT-PCR experiments performed on the same hippocampal neurons as those used in patch-clamp experiments, which showed a 10-fold lower level of ASIC2a mRNA compared to ASIC1a mRNA (N. Voilley, unpublished data). This suggests a higher probability of a heteromeric association between ASIC1a and ASIC2a subunits rather than a homomeric ASIC2a association, and that heterotetrameric ASIC1a+2a channels could involve more ASIC1a than ASIC2a subunits.

ASIC2b is known to induce a sustained outward-rectifying non-selective cationic current when associated with other ASIC subunits (Lingueglia *et al.* 1997). Since ASIC1a, ASIC2a and ASIC2b are expressed in hippocampal neurons (Price *et al.* 1996; Waldmann *et al.* 1996; Bassilana *et al.* 1997; Garcia-Anoveros *et al.* 1997), it is likely that heteromeric ASIC2b-containing channels are responsible for the sustained non-selective cation current recorded in hippocampal neurons. However, it is difficult to postulate the stoichiometry or the other subunits involved in these heteromeric channels (ASIC1a+2b, ASIC2a+2b or even ASIC1a+2a+2b). The participation of ASIC2b in the plateau phase is thus highly probable but the involvement of another channel type cannot be ruled out. Further biochemical evidence will be needed to confirm the presence and the molecular composition of functional heteromeric ASIC channels in hippocampal neurons.

Effects of extracellular pH variations on neuronal activity

There are situations in which acidic pH may lead to a decrease in neuronal excitability (Hsu *et al.* 2000). Protons inhibit NMDA currents and voltage-dependent Ca^{2+} currents, whereas $GABA_A$ currents are stimulated (Chesler & Kaila, 1992). In contrast, a rapid external acidification has been shown to excite a variety of neurons, due to the activation of ASIC-like currents (Gruol *et al.* 1980; Krishtal *et al.* 1987; Jarolimek *et al.* 1989; Walz, 1989; Chesler & Kaila, 1992; Varming, 1999). Neurons of rat ventral medulla oblongata have been shown to respond to small extracellular pH variations by a transient increase in AP frequency (Jarolimek *et al.* 1990). A rapid acid-triggered depolarization and the generation of spikes was also reported in mouse cortical spinal neurons (Gruol *et al.* 1980; Varming, 1999).

We show that ASIC-like current activation triggers a membrane depolarization and trains of APs in hippocampal neurons. Small pH changes, compatible with local transient acidifications reported in the CNS (Krishtal *et al.* 1987; Jarolimek *et al.* 1989; Chesler & Kaila, 1992; Miesenbock *et al.* 1998), can increase neuronal excitability by raising the

membrane potential to a level near the voltage-dependent Na^+ channel threshold.

Under certain pathological conditions, such as brain ischaemia, the local extracellular pH becomes quite acidic, reaching values around 6.5 (Ohno *et al.* 1989; Nedergaard *et al.* 1991). Activation of sustained ASIC currents is then expected to produce an intense firing of neurons which itself might contribute to neuronal death during an ischemic insult. Very recently, variations of expression of ASIC subunits has been related to physiopathological processes. Ischaemia induces the increase of ASIC2a expression in neurons of hippocampus and cortex (Johnson *et al.* 2001), whereas epilepsy induces a marked decrease in ASIC2b mRNA levels in all hippocampus areas and in ASIC1a mRNA levels in the CA1–2 fields (Biagini *et al.* 2001). Taken altogether, these results suggest an important role for ASIC subunits in both normal and pathological activity of hippocampus.

Effect of Zn^{2+} on ionic currents and neuronal excitability

Ever since the discovery that Zn^{2+} is present in large amounts in hippocampus, there has been intense speculation concerning a possible synaptic signalling role for this metal in brain function. In general, exogenous Zn^{2+} tends to lead to excitatory bursting (Harrison & Gibbons, 1994; Reece *et al.* 1994; Henze *et al.* 2000). Some of this excitatory effect is likely to be due to an inhibition of voltage-dependent K^+ channels as well as an inhibition of GABA channels and a potentiation of AMPA receptors. In contrast to its excitatory effects, Zn^{2+} blocks NMDA receptors (Harrison & Gibbons, 1994; Smart *et al.* 1994; Henze *et al.* 2000). Zn^{2+} was also reported to play a role in neurodegeneration associated with pathologies such as ischaemia and epilepsia (Harrison & Gibbons, 1994; Smart *et al.* 1994; Choi & Koh, 1998; Weiss *et al.* 2000).

We show that hippocampal ASIC-like current activation and the potentiation by Zn^{2+} modulates neuronal excitability by increasing the membrane depolarization induced by small pH changes. This effect was observed at Zn^{2+} concentrations compatible with the physiological range of synaptically released Zn^{2+} (100–300 μM) (Assaf & Chung, 1984; Howell *et al.* 1984; Smart *et al.* 1994; Budde *et al.* 1997; Weiss *et al.* 2000). ASIC channels might thus be important physiological targets of Zn^{2+} in the hippocampus.

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